Diet and blood pressure in 2.5-y-old Danish children

Janne Ulbak, Lotte Lauritzen, Harald S Hansen, and Kim F Michaelsen

ABSTRACT

Objective: The study’s aim was to investigate whether intakes of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) during lactation and current intakes of macronutrients affect blood pressure in 2.5-y-old Danish children.

Design: Mothers (n = 122) with low fish intakes were randomly assigned to receive supplementation with 4.5 g fish oil or olive oil/d during the first 4 mo of lactation. The trial also included 53 mothers with high fish intakes. One hundred five of these women’s children attended a 2.5-y follow-up examination at which anthropometric data and blood pressure were obtained. Mothers then kept a 7-d dietary record of food consumed by their children. A full set of data from 73 children was analyzed for effects of fish oil supplementation and cross-sectional correlations with current diet.

Results: We found no significant effect of the mothers’ fish oil intakes during the first 4 mo of lactation on the blood pressure of the children 2.5 y later. Greater protein intakes measured as a percentage of energy were associated cross-sectionally with significantly lower diastolic and systolic blood pressures in the children at age 2.5 y after control for outdoor temperature, age, sex, weight, and height (P = 0.028 and 0.035, respectively). Greater protein intakes measured as g/d were also associated with significantly lower systolic blood pressures (P = 0.008). A 1-SD increase in protein intake corresponded with a decrease of ≈3 mm Hg in systolic blood pressure.

Conclusion: The blood pressure of young Danish children was not significantly affected by intakes of n-3 LC-PUFAs via breast milk, but greater protein intakes at 2.5 y were associated with lower blood pressure. Am J Clin Nutr 2004;79:1095–102.

KEY WORDS Diet, protein, breast milk, long-chain n-3 polyunsaturated fatty acids, monounsaturated fatty acids, systolic blood pressure, diastolic blood pressure

INTRODUCTION

In the Western world, cardiovascular disease (CVD) is one of the most common causes of death, and high blood pressure is a major risk factor for CVD (1). Hypertension is unusual during childhood (2), but studies have shown that blood pressure tracks from early childhood into adulthood (3). Two studies indicated that intakes of n-3 polyunsaturated fatty acids (n-3 PUFAs) during pregnancy and infancy can program later blood pressure (4, 5). Studies in adults showed that diet can affect blood pressure (6) and suggested an inverse association between protein intakes and blood pressure (7). Two studies observed an association between breastfeeding in infancy and lower blood pressure during childhood (8, 9). Breast milk and infant formula deviate in content with respect to several components, among them PUFAs. Long-chain PUFAs (LC-PUFAs) are present in breast milk but not in most of the infant formulas currently available (10). The concentration of LC-PUFAs—especially n-3 LC-PUFAs—in breast milk depends on the maternal diet, and the overall ratio of n-3 PUFAs to n-6 PUFAs in breast milk in most countries is higher than that in infant formula (10). Perinatal n-3 PUFA deficiency has been shown to result in raised blood pressure later in life in rats, even if the animals were subsequently repleted with these fatty acids (4). Furthermore, dietary supplementation with n-3 LC-PUFAs was shown to cause a reduction in blood pressure in several trials in adults (11). A recent randomized trial showed that LC-PUFA supplementation of infant formula was associated with lower blood pressure in 6-y-old children (5).

We performed a randomized trial in which the n-3 LC-PUFA intakes of breastfed infants were increased via fish oil (FO) supplementation to their mothers during the first 4 mo of lactation. The trial was designed to investigate the effects on growth and development during the first year of life. Long-term effects on growth and health—eg, on blood pressure—were investigated at a follow-up visit when the children were 2.5 y old.

The current study also provided an opportunity to examine the relation between current diet and blood pressure in young children. Few studies have investigated the relation between macronutrients and blood pressure in prepubertal children, and only one of those studies concerned 2-y-old children (12), whereas the others concerned children aged 8–11 y (13–15). Greater intakes of energy, fat, and carbohydrates were associated with lower blood pressure in 2-y-old children (12). The associations were, however, sex specific: the energy and fat association was observed only in boys, and the carbohydrate association was observed only in girls (12). Greater intakes of both fat and carbohydrates were shown to be associated with higher blood pressure.
in a mixed-sex group of older children (14). No association was observed between protein intakes and blood pressure in 2-y-old children (12), but greater protein intakes were associated with lower blood pressure in older children (13, 14), just as in adults. These studies provide no final conclusions about how macronutrients affect blood pressure in young children.

The aim of this study was to ascertain whether maternal FO supplementation during lactation would affect later blood pressure. We also analyzed whether there was a difference in blood pressure between the children of mothers with habitual high fish intakes and the children of mothers with habitual low fish intakes. Furthermore, the study aimed to investigate the relation between blood pressure and current intakes of macronutrients in children in the third year of life.

**SUBJECTS AND METHODS**

**Subjects**

The protocols for the intervention trial and follow-up study were approved by the scientific and ethical committee of Copenhagen and Frederiksberg (KF 01–300/98 and KF 01–183/01). Both parents of all participating children gave written informed consent to participate after the study had been explained to them orally and in writing.

Participants were selected from among women recruited for the ongoing Danish National Birth Cohort (DNBC; 16). In the 25th week of gestation, the diets of all members of the cohort were ascertained by using a comprehensive 300-item food-frequency questionnaire (FFQ) that asked about their diet for the previous 4 wk. Consumption of n -3 LC-PUFAs in g/d was estimated by using assumptions about portion sizes and the nutrient content of foods taken from tables produced by the Danish Food Agency. During the period from April 1999 to February 2000, 919 pregnant women from the greater Copenhagen area who had a fish intake below the 44th percentile (<0.40 g n -3 LC-PUFAs/d) and who were in the 8th month of gestation were invited to participate in the trial. We also invited women with a fish intake above the 74th percentile (>0.82 g n -3 LC-PUFAs/d) to participate in the study as a high fish intake reference group. We received a positive response from 273 women, of whom 211 (147 with a low and 64 with a high fish intake) fit the other inclusion criteria: their pregnancy was uncomplicated, and they had a BMI <30, no metabolic disorders, and an intention to breastfeed for ≥4 mo. We also required the women to begin taking the supplements within 2 wk after the birth of their infants. In addition, participation in the study required the women’s newborns to be healthy (no admission to a neonatal department), term, singleton infants with normal weight for gestation and an Apgar score >7 at 5 min after delivery. Of the women in the low and high fish intake groups, 122 and 53, respectively, fulfilled all of these criteria.

**Trial and supplements**

After the birth of their infants, the women with a low fish intake were randomly allocated to receive supplementation with FO or olive oil in blocks of 2 and in 5 strata according to the mean education level of both parents of the child (17). The parents were asked about their education level, which was then scored from 1 to 8 (1 = primary school, 8 = master’s degree or higher) according to the Official Danish Classification of Education from 1994.

Women were allocated to the FO group (n = 62) or the olive oil control group (n = 60). Investigators and families were blinded to the randomization throughout the first year of the child’s life.

Both supplements were given as microencapsulated oils concealed in 2 müsli bars (Halo Foods Ltd, Tywyn Gwynedd, United Kingdom) that were consumed daily during the first 4 mo of lactation. The FO group received 17 g/d of a 1:2 mixture of 2 microencapsulated FOs, with 47 mg eicosapentaenoic acid (EPA)/g and 32 mg docosahexaenoic acid (DHA)/g and with 16 mg EPA/g and 75 mg DHA/g, respectively (Dry n -3 18:12 and Dry n -3 5:25, respectively; BASF Health and Nutrition A/S, Ballerup, Denmark). This mixture provided 4.5 g FO and 1.5 g n -3 LC-PUFA, which is equivalent to the habitual intake of the women in the population with the highest fish intake (>90th percentile). The control olive oil group was given a similar amount of microencapsulated olive oil. As an alternative, women were offered the supplements in homemade cookies or in capsules if they disliked the müsli bars. The homemade cookies were made in the department kitchen with microencapsulated oils in amounts similar to the amounts in the müsli bars. To obtain an approximately similar supplementation with capsules, the control group was given four 1000-mg olive oil capsules, and the FO group was given six 500-mg capsules/d with a high concentration of DHA and one 1000-mg capsule/d with a low concentration of DHA (all capsules were a gift from Lupe/ProNova Biocare, Lysaker, Norway). The 4 g FO supplied 1.4 g n -3 LC-PUFAs (358 mg EPA and 992 mg DHA). The distribution of müsli bars, cookies, and capsules among participants in the FO and olive oil groups was identical: 10% of the women received their entire supplementation in capsules, and 60% of the women had only müsli bars or cookies. The smaller amount of oil supplied by the capsules was taken into account in the calculation of overall supplement compliance, which is expressed as the percentage of oil taken relative to the intended dose of 2 müsli bars/d. The overall mean self-reported compliance in both groups was 88 ± 1% (n = 99) during the entire 4-mo supplementation period. One hundred mothers from the low fish intake group and 50 mothers from the high fish intake reference group remained in the study throughout the 4-mo study period.

Seventy-one percent of the mothers complied with the intention to exclusively breastfeed for ≥4 mo. Those who did not comply were not excluded from the trial or the analysis, but we estimated the degree to which breast milk supplied the energy needs of the infant from the amount of formula and complementary food ingested. Among those infants who were not exclusively breastfed, breast milk supplied >90–99%, 75–90%, 50–<75%, and <50% of the energy needs of 11%, 6%, 2%, and 10% of the infants, respectively. The degree of breastfeeding was taken into account in multiple regression analyses of the outcome.

**Follow-up study**

When the children were 2.5 y old, all 150 families were invited to participate in the follow-up study, which was carried out from November 2001 to September 2002 at the Department of Human Nutrition, Frederiksberg, Denmark. Of the 150 families who completed the 4-mo study period, 11 were lost to follow-up, 6 had moved away from the area, 15 did not wish to attend the follow-up examination for various personal reasons, and 13 did not give any reason for their lack of participation. One hundred five families agreed to participate in the follow-up study. Four of
Anthropometric measurements

The infants’ height and weight at birth were taken from the hospital journal. The height at 2.5 y was measured in barefoot children to 0.1 cm by using a stadiometer (model 28.P.4; CMS Weighing Equipment Ltd, London). Body weight was measured to 0.1 kg on a digital scale (Lindeltronic 8000; Samhall Lavi AB, Kristianstad, Sweden).

Blood pressure

The children’s blood pressure was assessed by using an automatic device (model 506N; Criticare Systems Inc, Waukesha, WI) during cuff inflation. Two cuff sizes were used, depending on the size of the child’s arm: one for an arm circumference of 10–19 cm and one for an arm circumference of 18–26 cm. The child was sitting, and the investigator supported the cuffed arm while the blood pressure was measured. Blood pressure was measured twice, shortly after arrival and at the end of the visit, with ≈1 h between measurements. Before the second measurement, the child generally rested for 30 min by sitting on an examination couch and watching a video. The second measurement was used for analysis.

There was a high degree of correlation between the first and second measurements of blood pressure (r = 0.988 and r = 0.995 for diastolic and systolic blood pressure, respectively, for regression through origin, both with no constant bias and P < 0.001). The blood pressure of 6 children was measured twice at the end of the visit. The cuff was removed between those 2 measurements. CVs were calculated from these 2 measurements as well as from the 2 measurements taken an hour apart in all children. In both cases, CVs were ≈10% for diastolic and systolic blood pressure.

The average maximum outdoor temperature during the week of the follow-up examination was used to control for variations in blood pressure caused by temperature, which previous studies had reported finding (18, 19). The temperatures were obtained from the Danish Meteorological Institute, Copenhagen.

Diet

The parents received written and oral instructions on recording the food intakes of the children. The diet of the children was recorded for 7 consecutive days by using a coded dietary questionnaire adapted from the questionnaire used in the Danish National Food Surveys (20). For each meal during the day, the questionnaire had 445 categories referring to all normal types of foods, drinks, and dishes eaten by Danish children aged 0.5–4 y. A parent completed the questionnaire by indicating which kinds of foods were eaten by the child during each of the 7 d. The amount of food was given in household measures or standard portion sizes estimated from a collection of pictures. Food intakes in the day-care institution were recorded in household measurements and transferred to the diet questionnaire by the parents every night. The questionnaire also had open fields that the parent could use if the child had eaten or drunk something that did not fit into the coded categories. All data from the questionnaires were entered into the GIES computer program (version 0.9; Danish Veterinary and Food Administration, Søborg, Denmark) that contained standard recipes for all coded dishes and standard serving sizes. This information was used to convert into grams the household measures of 296 unique food types. The average daily nutrient intakes of the children were calculated from the resulting diet by using the GIES computerized nutrient database (version 0.9).

Statistical analysis

All results are given as means ± SEs. All variables were normally distributed, except length at birth and intakes of fat and saturated fatty acids (g/d). A nonparametric test for comparisons (Mann-Whitney U test) was used to analyze those variables, and P values for these measurements were verified by regression analysis with log-transformed values. Student’s t test was used to compare the follow-up group with the children who did not participate in follow-up and to compare the blood pressures of the children in the 2 randomized groups. Differences in the dietary intakes of boys and girls were also examined by using Student’s t test.

Relations between blood pressure and independent variables were assessed by using Pearson’s correlation and multiple linear regressions. Independent dietary variables included energy, macronutrients [protein, total fat, saturated fatty acids, monounsaturated fatty acids (MUFAs), PUFAs, and carbohydrates], cholesterol, and fiber. All analyses of possible associations between blood pressure and dietary factors were controlled for outdoor temperature. Furthermore, associations were controlled for weight and height, and multiple regressions were controlled for age and sex, although neither of those factors was significantly associated with diastolic or systolic blood pressure. Age was used as a controlling variable because the literature shows a relation between age and blood pressure (2, 21). Sex was included because a previous study on macronutrients and blood pressure in 2-y-old children found different results for boys and girls (12). All multiple regression analyses of associations between nutrients and blood pressure were also performed by including a sex × nutrient interaction term. If the interaction term had a significant effect in this analysis, then the multiple regression analysis was performed separately in the 2 sexes. All data were analyzed by using SPSS for WINDOWS software (version 11.0; SPSS Inc, Chicago).

RESULTS

Weight was measured in all 101 children, and height was measured in 100 children. Blood pressure was measured successfully in 82 children; the rest were uncooperative, and complete diet records were returned from 89 families. Seventy-three children had complete data sets (except for a single measure of height), and all results are based on data from these children. The 73 children with full follow-up data were similar to the children with no follow-up data with respect to gestational age, weight and length at birth, and degree of breastfeeding (data not shown). There was, however, a significantly higher follow-up rate for boys than for girls (P = 0.039), and the follow-up group had significantly better compliance in the intervention trial than did the group without follow-up data (90% and 86%, respectively).
Bivariate analysis of control values and blood pressure in 2.5-y-old children

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure in the entire group of 2.5-\text{-}y\text{-}old children and in subgroups divided according to maternal intakes of n – 3 long\text{-}chain polyunsaturated fatty acids during lactation(^1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Diastolic (mm Hg)</th>
<th>Systolic (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x} \pm SE)</td>
<td>66 ± 1</td>
<td>109 ± 1</td>
</tr>
<tr>
<td>Range</td>
<td>44–92(54–81)</td>
<td>88–134(96–123)</td>
</tr>
<tr>
<td>Fish oil group (n = 30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x} \pm SE)</td>
<td>67 ± 2</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>Range</td>
<td>44–92</td>
<td>88–134</td>
</tr>
<tr>
<td>Olive oil group (n = 22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x} \pm SE)</td>
<td>67 ± 2</td>
<td>108 ± 2</td>
</tr>
<tr>
<td>Range</td>
<td>50–86</td>
<td>90–121</td>
</tr>
<tr>
<td>High fish intake reference group (n = 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x} \pm SE)</td>
<td>63 ± 2</td>
<td>108 ± 2</td>
</tr>
<tr>
<td>Range</td>
<td>46–77</td>
<td>95–124</td>
</tr>
</tbody>
</table>

\(^1\) There were no significant differences between the groups.
\(^2\) Overall range; 10th–90th percentile range in parentheses.

Forty-six boys and 27 girls came to the follow-up visit when they were 31.7 ± 0.1 mo old. Their height, weight, and BMI were 92.7 ± 0.4 cm, 14.0 ± 0.1 kg, and 16.3 ± 0.1, respectively. They were born after 40.2 ± 0.1 wk of gestation with a mean weight of 3.63 ± 0.05 kg, length of 52.4 ± 0.3 cm, and ponderal index of 25.2 ± 0.3 kg/m\(^3\).

The blood pressures of the entire study group of children and of the different dietary subgroups are given in Table 1. The systolic blood pressure differed by 4 ± 3 mm Hg between the 2 randomized groups. Children of mothers with habitual high fish intake had a diastolic blood pressure 4 mm Hg lower than did children of mothers with habitual low fish intake. However, none of these differences were significant \((P = 0.199\) and \(P = 0.104\), respectively); after control for outdoor temperature, sex, age, weight, and height, the values were \(P = 0.4\) and \(P = 0.2\), respectively. FO supplementation of the lactating mothers \((n = 46)\) resulted in a substantially greater n – 3 LC-PUFA content in breast milk during the 4 mo of lactation than in the breast milk of mothers in the control group \((n = 45)\): 1.93 ± 0.13% and 0.76 ± 0.05%, respectively \((P < 0.001)\).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Diastolic blood pressure</th>
<th>Systolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(b \pm SE)</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>0.005</td>
<td>0.05 ± 1.26</td>
</tr>
<tr>
<td>Sex(^2)</td>
<td>0.082</td>
<td>1.70 ± 2.44</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.443</td>
<td>-3.52 ± 0.85</td>
</tr>
<tr>
<td>Height (cm)(^3)</td>
<td>-0.289</td>
<td>-0.95 ± 0.38</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>-0.290</td>
<td>-2.60 ± 1.03</td>
</tr>
<tr>
<td>Weight at birth (kg)</td>
<td>-0.128</td>
<td>-2.96 ± 2.73</td>
</tr>
<tr>
<td>Length at birth (cm)</td>
<td>-0.117</td>
<td>-0.55 ± 0.55</td>
</tr>
<tr>
<td>Ponderal index at birth (kg/m(^3))</td>
<td>-0.032</td>
<td>-0.15 ± 0.56</td>
</tr>
<tr>
<td>Outdoor temperature (°C)</td>
<td>-0.421</td>
<td>-0.59 ± 0.15</td>
</tr>
<tr>
<td>Duration of breastfeeding (mo)</td>
<td>-0.032</td>
<td>-0.08 ± 0.28</td>
</tr>
</tbody>
</table>

\(^1\) Total \(n = 73\).
\(^2\) Female = 1, male = 2.
\(^3\) \(n = 72\).
on 11 June 2018

1B). No effect of the sex × protein intake interaction was observed on systolic blood pressure or between blood pressure and protein intake expressed as percentage of energy or intake of other nutrients.

**DISCUSSION**

Our most consistent and interesting finding was a negative association between protein intake and both systolic and diastolic blood pressure. Neither the FO supplementation nor habitual fish intake of the mother had an effect on the child’s blood pressure.

Protein intake expressed as percentage of energy was associated with lower diastolic and systolic blood pressure, and protein intake expressed as g/d was associated with systolic blood pressure, and protein intake expressed as g/d was assessed the effect in children of the same age group, and it did not find a significant association (12).

Our study population was very homogenous, with a narrow age range, a high degree of breastfeeding, and birth weight and gestational age within the normal range. This homogeneity gives an increased strength for identifying associations between diet and blood pressure. The children in this study consumed an average of 11.9% of energy as protein, and ≈90% of the children had protein intakes within the recommended range, which in Scandinavia overall is 10–15% of energy for children aged 1–3 y (22). A large population survey found the protein intake of Danish children at the age of 1–3 y to be 13.5% of energy (20). The protein intake of young Danish children is in agreement with that in other reports on protein intake in 2 y-old children (12). There is always a possibility for errors in dietary assessments, either because the diet in the period of assessment is consciously or unconsciously improved or because the diet recorded does not

**TABLE 3**

Dietary intakes for 7 d in children at 2.5 y

<table>
<thead>
<tr>
<th>Energy intake&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Intake&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of energy</td>
</tr>
<tr>
<td></td>
<td>10th percentile</td>
</tr>
<tr>
<td></td>
<td>10th percentile</td>
</tr>
<tr>
<td>Protein</td>
<td>11.9 ± 0.2&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>36.1 ± 0.5</td>
</tr>
<tr>
<td>Saturated</td>
<td>15.7 ± 0.3</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>10.7 ± 0.2</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>4.06 ± 0.07</td>
</tr>
<tr>
<td>n − 3 PUFAs</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>—</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>52.1 ± 0.5</td>
</tr>
<tr>
<td>Sugar</td>
<td>12.0 ± 0.4</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>1</sup> n = 73. PUFAs, polyunsaturated fatty acids.

<sup>2</sup> The mean total energy intake was 6074 ± 136 kJ.

<sup>3</sup> Significant difference (P = 0.006) between boys (11.5 ± 0.2% of energy) and girls (12.5 ± 0.2% of energy).

**TABLE 4**

Dietary intakes and blood pressure by multiple regression analysis after control for current weight, height, age, sex, and outside temperature<sup>1</sup>

<table>
<thead>
<tr>
<th></th>
<th>Diastolic blood pressure</th>
<th>Systolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b ± SE</td>
<td>P</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>−0.60 ± 0.74</td>
<td>0.035</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>−0.05 ± 0.26</td>
<td>0.834</td>
</tr>
<tr>
<td>Saturated</td>
<td>−0.22 ± 0.41</td>
<td>0.586</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>−0.53 ± 0.39</td>
<td>0.447</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>0.28 ± 1.74</td>
<td>0.875</td>
</tr>
<tr>
<td>n − 3 PUFAs</td>
<td>−0.17 ± 7.13</td>
<td>0.390</td>
</tr>
<tr>
<td>Carbohydrates (% of energy)</td>
<td>0.23 ± 0.26</td>
<td>0.365</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>−0.0001 ± 0.0001</td>
<td>0.923</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>−0.18 ± 0.13</td>
<td>0.182</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>0.02 ± 0.08</td>
<td>0.790</td>
</tr>
<tr>
<td>Saturated (g/d)</td>
<td>−0.08 ± 0.16</td>
<td>0.615</td>
</tr>
<tr>
<td>Monounsaturated (g/d)</td>
<td>−0.13 ± 0.24</td>
<td>0.584</td>
</tr>
<tr>
<td>Polyunsaturated (g/d)</td>
<td>0.01 ± 0.66</td>
<td>0.986</td>
</tr>
<tr>
<td>n − 3 PUFAs (g/d)</td>
<td>−0.003 ± 0.003</td>
<td>0.448</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>−0.01 ± 0.02</td>
<td>0.563</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>0.01 ± 0.03</td>
<td>0.695</td>
</tr>
<tr>
<td>Dietary fiber (g/d)</td>
<td>0.18 ± 0.32</td>
<td>0.579</td>
</tr>
</tbody>
</table>

<sup>1</sup> n = 72. PUFAs, polyunsaturated fatty acids.
Blood pressure (mm Hg) as a function of protein intake (in g/d). Both vari-
ables are adjusted for outdoor temperature, weight, height, and age. The symbols
represent data from boys (●) and girls (○). The fitted regression line with
95% CIs is based on data from both sexes and has a \( \hat{\beta} \pm \text{SE} \) correlation
coefficient of \(-0.325 (\hat{\beta} = -0.39 \pm 0.13, P = 0.005, n = 72)\). (B) Diastolic
blood pressure (mm Hg) as a function of protein intake (in g/d). Both vari-
ables have been adjusted, and symbols represent data from boys (●) and girls
(○). Regression lines have been made separately for the boys \((r = -0.348,
\hat{\beta} = -0.40 \pm 0.16, P = 0.019; n = 45; - - -)\) and girls \((r = 0.115, \hat{\beta} = 0.11
\pm 0.20, P = 0.568; n = 27; - - -)\) because there was a significant effect of a
protein intake \( \times \) sex interaction on diastolic blood pressure.

FIGURE 1. Blood pressure versus protein intake. (A) Systolic blood
pressure (mm Hg) as a function of protein intake (in g/d). Both variables are
adjusted for outdoor temperature, weight, height, and age. The symbols
represent data from boys (●) and girls (○). The fitted regression line with
95% CIs is based on data from both sexes and has a \( \hat{\beta} \pm \text{SE} \) correlation
coefficient of \(-0.325 (\hat{\beta} = -0.39 \pm 0.13, P = 0.005, n = 72)\). (B) Diastolic
blood pressure (mm Hg) as a function of protein intake (in g/d). Both vari-
ables have been adjusted, and symbols represent data from boys (●) and girls
(○). Regression lines have been made separately for the boys \((r = -0.348,
\hat{\beta} = -0.40 \pm 0.16, P = 0.019; n = 45; - - -)\) and girls \((r = 0.115, \hat{\beta} = 0.11
\pm 0.20, P = 0.568; n = 27; - - -)\) because there was a significant effect of a
protein intake \( \times \) sex interaction on diastolic blood pressure.

reflect the actual intakes. However, these problems are more
predominant in adolescents or adults (23), and recordings of
children’s diets tend to be more valid (24). Furthermore, we used
a relatively long period (7 d) for assessing the diet. It is possible
that protein intake may be associated with the socioeconomic
status of the children, which would then be a possible confound-
ing factor. However, the results of the multiple regression anal-
ysis were not affected by the inclusion of parental education.

A previous study of formula-fed infants reported that n–3
LC-PUFA supplementation during first year of life reduced
blood pressure in the children at age 6 y (5), but we observed no
such effect in the present study. FO supplementation was shown
to result in a significant reduction in blood pressure in adults (11).
The intakes of n–3 LC-PUFAs in the Danish population in
general are high compared with those in many other populations.
Furthermore, the children in our study were almost completely
breastfed (breast milk was estimated to provide an average of
91% of their diet during the first 4 mo of life). In the control olive
oil group, n–3 LC-PUFAs contributed an average of 0.76% of
the fatty acids in the breast milk, which resulted in a total n–3
PUFA intake of \( \approx 1\% \) of energy. The infant formula that was used in
the control group of the study by Forsyth et al (5) had a very low
n–3 PUFA content (25) that did not comply with the recom-
ended supply of 0.5% of energy as n–3 PUFAs (22). The
rodent study that originally showed a long-term effect of early
n–3 PUFA intake on blood pressure investigated that effect in
the progeny of mothers who were made deficient in n–3 PUFA
during pregnancy and lactation (4). The lack of an effect of the
early n–3 PUFA intakes in the present study, at least with respect
to future increases in blood pressure, may therefore be due to the
fact that all children in our study had sufficient n–3 PUFA
intakes in infancy. Unlike infants, young Danish children do not
have a very high intake of PUFAs (20). In the present study, we
found that their intake of n–3 PUFAs was 0.5–0.9% of energy,
which just meets the current Scandinavian recommendations for
the minimal n–3 PUFA intake in that age group (22). We found
no significant associations between the current n–3 PUFA in-
takes of the children and their blood pressure.

The average diastolic and systolic blood pressures of the chil-
dren in this study are high compared with those found in other
studies of children of a similar age (2, 21). However, none of
those studies used the same device for measuring blood pressure,
which makes it difficult to compare values directly, because
different devices tend to measure blood pressure differently (26,
27). Oscillometric techniques such as the technique used in this
study have been shown to give blood pressure readings 5–10 mm
Hg higher than those obtained in the same subjects with the use of
other methods (27). Thus, it seems plausible that the high blood
pressures in the present study may have a methodologic explanation.
A less likely explanation could be that the children were not fully
rested before or during the blood pressure assessment.

Neither birth size, duration of breastfeeding, nor age was
found to have an effect on blood pressure in the present study.
Several studies found a relation between size at birth and blood
pressure in the third year of life (28, 29) or later in life (30). A
negative association between the duration of breastfeeding and
later blood pressure was also shown (8, 9, 12). Blood pressure
was shown in several studies to be higher in older children than
in younger children (2). We found that greater weight, height, and
BMI were linked to lower diastolic blood pressure. This finding
was unexpected because the literature has reported positive as-

cociations (2, 31, 32). Variations in birth size, duration of breast-

feeding, and age span of the children were limited in the present
study by the study’s design. The lack of variation in these vari-
ables could explain why no associations were seen in this study.

There was no difference between the sexes in either diastolic
or systolic blood pressure, which agreed with the findings of
other studies in this age group (31, 33). However, we found a
significant effect of the sex \( \times \) protein intake (g/d) interaction
on diastolic blood pressure, and, when the association between
protein intake and diastolic blood pressure was analyzed in the sexes
separately, it was significant only in boys. Studies in children of the same age as those in the current study (12) and in older children (13) also found different results in boys and girls. The sex difference in our study may be explained by the fact that boys outnumbered girls, and thus analysis in girls alone had less statistical power, although no trends were found to support this possibility (data not shown). Thus, we cannot exclude the possibility that the difference could be due to physiologic differences between the sexes.

It has been proposed that the mechanism behind the association between protein and blood pressure could be the vasodilating properties of certain amino acids, mediated through an increased production of nitric oxide (34). Intravenous or intraperitoneal administration of some amino acids was shown to cause a lowering of blood pressure (35). However, this effect was observed at concentrations that are higher than the ones to which dietary amino acids are able to give rise. It is also possible that the effect of protein may be due to other nutritional components that vary with the protein intakes. The children in the study received 33% of their protein from milk or dairy products (data not shown). Thus, the effect of protein could be related to other bioactive components in the milk. Preus et al (34) suggested that protein’s effect of reducing blood pressure may have to do with the relation between intakes of protein, fat, and carbohydrates, and that it may be the intakes of these other macronutrients that have an effect on blood pressure.

Other nutrients that covariate with protein intake, e.g., salt, could also account for the association with blood pressure. Salt intake was, however, positively correlated with protein intake in the current study. All reports on the association between salt intake and blood pressure are positive (36). Thus, covariance of salt and protein does not seem to be a plausible explanation for the association between blood pressure and protein. On the contrary, we would expect that salt might cancel out some of the association between blood pressure and protein seen in the present study. We found a negative relation between MUFA intakes (g/d and % of energy) and systolic blood pressure, but no relation with diastolic blood pressure was observed, and no association between blood pressure and intakes of fat, saturated fatty acids, PUFAs, or cholesterol was found. Simons-Morton et al (14) also found a relation between MUFA intakes and blood pressure in children, but this was a positive relation. There are studies in adults, however, that found negative associations (37).

In conclusion, our most important finding was that protein intake was associated with lower blood pressure. This finding should not be used to recommend a higher protein intake, however, because other studies suggested adverse effects of a high protein intake early in life (38–40). It would be interesting to see whether intervention studies could confirm the association between protein intake and blood pressure and whether studies could identify the effective mechanisms.

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